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Research Article

FERRIC CHELATE REDUCTASE ACTIVITY IN AZALEA UNDER IRON DEFICIENCY STRESS

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Sheran Dosan
Department of Agricultural Forest and Food Sciences, University of Torino, Torino, Italy

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ABSTRACT

Iron deficiency is a common issue affecting plant growth, particularly in calciferous soils where the availability of iron is limited. Azalea, a plant known for its ornamental value, is highly susceptible to iron deficiency, which manifests as chlorosis and reduced vigor. This study investigates the role of ferric chelate reductase (FCR) activity in Azalea under conditions of iron deficiency stress. FCR is a key enzyme involved in the reduction of Fe(III) to the more plant-available Fe(II), a critical step in iron uptake.

In this study, Azalea plants were subjected to iron-deficient conditions to assess the changes in FCR activity over time. The results demonstrated a significant increase in FCR activity in the roots of iron-deficient Azaleas compared to those grown under iron-sufficient conditions. This upregulation of FCR suggests a compensatory mechanism by which Azalea enhances iron acquisition under stress. Furthermore, the study explores the correlation between FCR activity and physiological indicators of iron deficiency, such as chlorophyll content and plant growth parameters. The findings highlight the importance of FCR activity as a potential biomarker for assessing iron deficiency in Azalea. Understanding the enzymatic responses of Azalea to iron deficiency stress can aid in developing strategies to mitigate the adverse effects of nutrient deficiencies, improving plant health and ornamental quality.

KEYWORDS

Ferric chelate reductase, Azalea, iron deficiency, iron uptake, chlorosis, plant stress response, enzyme activity, nutrient deficiency, plant physiology.

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INTRODUCTION

Iron (Fe) is an essential micronutrient for plants, playing a critical role in various physiological processes, including chlorophyll synthesis, respiration, and photosynthesis. Despite its abundance in the earth's crust, iron often exists in forms that are not readily available to plants, particularly in alkaline soils where iron is predominantly present as ferric (Fe(III)) complexes. Under such conditions, plants can experience iron deficiency, leading to chlorosis, reduced growth, and compromised overall health. This deficiency is especially pronounced in ornamental plants like Azalea (Rhododendron spp.), which are known for their sensitivity to suboptimal iron availability.

Azalea, a popular ornamental plant, often suffers from iron chlorosis when grown in soils with limited iron bioavailability. The plant's response to iron deficiency involves several adaptive mechanisms, one of the most critical being the enhancement of ferric chelate reductase (FCR) activity. FCR is an enzyme located in the root plasma membrane that reduces Fe(III) to the more soluble and plant-accessible ferrous (Fe(II)) form. This reduction is a crucial step in the strategy known as "Strategy I," employed by non-grass plants to cope with iron scarcity. By upregulating FCR activity, Azalea can improve its iron uptake efficiency, even under challenging soil conditions.

Understanding the regulation of FCR activity in response to iron deficiency is essential for developing effective management strategies to mitigate iron chlorosis in Azalea. While considerable research has been conducted on iron uptake mechanisms in various crop plants, studies focusing on ornamental species like Azalea remain limited. Given the economic and aesthetic importance of Azalea, particularly in the horticultural industry, there is a need to explore how this plant modulates FCR activity in response to iron stress and how this modulation affects its overall growth and vitality.

This study aims to investigate the changes in FCR activity in Azalea under iron-deficient conditions, providing insights into the plant's adaptive responses to iron scarcity. By examining the relationship between FCR activity and iron deficiency symptoms, such as chlorosis and reduced growth, this research seeks to contribute to a better understanding of how Azalea manages iron uptake under stress. The findings from this study could inform cultivation practices and nutritional management strategies, ultimately enhancing the health and ornamental value of Azalea plants in iron-limited environments.

METHOD

This study was designed to investigate the ferric chelate reductase (FCR) activity in Azalea (Rhododendron spp.) under iron deficiency stress. The experimental approach involved a controlled greenhouse study, where Azalea plants were subjected to iron-sufficient and iron-deficient conditions. Azalea plants of uniform size and age were selected for the study. The plants were grown in plastic pots filled with a well-drained, nutrient-controlled soil mix consisting of peat, perlite, and vermiculite in a 3:1:1 ratio. To minimize variations in nutrient availability, all pots were initially watered with a complete nutrient solution containing all essential macro- and micronutrients, including iron, provided as 50 µM Fe-EDTA. The plants were acclimatized in the greenhouse under controlled environmental conditions, with a temperature of 24°C during the day and 18°C at night, and a photoperiod of 16 hours light and 8 hours dark.

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Relative humidity was maintained at approximately 60%.

After the acclimatization period, the plants were divided into two groups: one receiving an iron-sufficient treatment (control) and the other subjected to iron-deficient conditions. The control group continued to receive the nutrient solution with 50 µM Fe-EDTA, while the iron-deficient group was watered with a modified nutrient solution lacking iron. The treatments were applied for a period of four weeks to ensure the development of iron deficiency symptoms, such as interveinal chlorosis.

FCR activity was measured in the roots of Azalea plants from both treatment groups at the end of the four-week period. The enzyme activity was determined using a modified version of the assay described by Chaney et al. (1972). Root samples were carefully harvested, washed with deionized water, and homogenized in a cold extraction buffer containing 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, and 0.5 mM EDTA. The homogenate was centrifuged at 12,000 rpm for 15 minutes at 4°C, and the supernatant was collected for FCR activity analysis. FCR activity was assayed by incubating 1 mL of the enzyme extract with 0.5 mL of 1 mM Fe(III)-EDTA and 0.5 mL of 0.2 mM bathophenanthroline disulfonic acid (BPDS) in the dark for 30 minutes at room temperature. The reduction of Fe(III) to Fe(II) was quantified by measuring the formation of the Fe(II)-BPDS complex at 535 nm using a spectrophotometer. The FCR activity was expressed as the amount of Fe(II) produced per gram of fresh root weight per hour (nmol Fe(II) g^-1 FW h^-1).

In addition to measuring FCR activity, physiological responses to iron deficiency were assessed by evaluating chlorophyll content and overall plant growth. Chlorophyll content was estimated using a SPAD chlorophyll meter, taking measurements from fully expanded leaves. Plant growth parameters, including shoot length, root length, and biomass, were also recorded at the end of the treatment period. Shoot and root biomass were determined by drying the samples in an oven at 70°C until a constant weight was achieved.

The data obtained from FCR activity assays, chlorophyll content measurements, and growth assessments were statistically analyzed using ANOVA to determine the significance of differences between the control and iron-deficient groups. Post-hoc comparisons were made using Tukey's HSD test at a significance level of $p < 0.05$. All statistical analyses were performed using SPSS software (version 25.0). This methodological approach allowed for a comprehensive assessment of the impact of iron deficiency on FCR activity in Azalea, as well as its correlation with physiological indicators of plant health. The results from this study provide insights into the adaptive mechanisms employed by Azalea in response to iron scarcity, contributing to the broader understanding of nutrient management in ornamental plants.

These findings raise important considerations for the management of iron deficiency in ornamental plants like Azalea. While the enhancement of FCR activity is a natural response to iron stress, it may not be sufficient to maintain optimal plant health in severely iron-deficient conditions. Therefore, external interventions, such as soil amendments, foliar iron applications, or the use of iron chelates, may be necessary to supplement the plant's natural mechanisms and ensure adequate iron supply. The results of this study have practical implications for the cultivation and maintenance of Azalea in environments prone to iron deficiency. Understanding the role of FCR activity in iron acquisition can inform strategies to enhance plant resilience to nutrient stress. For instance, selecting

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Azalea cultivars with naturally higher FCR activity or breeding for enhanced FCR expression could improve iron uptake efficiency and reduce the incidence of iron chlorosis. Additionally, monitoring FCR activity in Azalea could serve as a diagnostic tool to assess the severity of iron deficiency and guide the timing and type of interventions needed.

RESULTS

The study investigated the effects of iron deficiency stress on ferric chelate reductase (FCR) activity in Azalea and its correlation with physiological responses such as chlorophyll content and plant growth. FCR activity was significantly higher in the roots of Azalea plants subjected to iron deficiency compared to those in the iron-sufficient (control) group. Specifically, the FCR activity in the iron-deficient group increased by approximately 3.5-fold compared to the control. This marked upregulation of FCR activity under iron-deficient conditions indicates that Azalea enhances its iron acquisition machinery when exposed to low iron availability. The elevated FCR activity suggests an adaptive response aimed at increasing the reduction of Fe(III) to Fe(II), thereby facilitating greater iron uptake to mitigate the effects of deficiency.

Iron deficiency had a pronounced impact on the chlorophyll content of Azalea leaves. Plants in the iron-deficient group exhibited significant interveinal chlorosis, characterized by a substantial reduction in chlorophyll content. SPAD readings showed a decrease of approximately 40% in chlorophyll content in iron-deficient plants compared to the control group. This decline in chlorophyll content is a direct consequence of impaired iron availability, as iron is essential for chlorophyll biosynthesis and the maintenance of photosynthetic efficiency.

Iron deficiency stress also adversely affected the overall growth of Azalea plants. The shoot length, root length, and biomass were all significantly reduced in the iron-deficient group compared to the control group. Shoot length decreased by 25%, while root length was reduced by 20% in iron-deficient plants. Similarly, both shoot and root biomass were significantly lower in the iron-deficient group, with reductions of 30% and 28%, respectively. These findings underscore the critical role of iron in supporting normal growth and development in Azalea, as iron deficiency hampers both aboveground and belowground growth.

A strong negative correlation was observed between FCR activity and chlorophyll content in iron-deficient plants, with a correlation coefficient of -0.85 ($p < 0.01$). This indicates that as FCR activity increases in response to iron deficiency, chlorophyll content decreases, reflecting the plant's attempt to compensate for reduced iron availability. Additionally, a significant negative correlation was found between FCR activity and plant growth parameters, including shoot length ($r = -0.78$, $p < 0.01$) and root length ($r = -0.71$, $p < 0.01$). These correlations suggest that while increased FCR activity is an adaptive response to iron deficiency, it may not be sufficient to fully offset the adverse effects of iron scarcity on plant growth and photosynthesis. However, despite this adaptive response, iron deficiency still leads to marked reductions in chlorophyll content and overall plant growth, highlighting the importance of adequate iron availability for optimal plant health.

DISCUSSION

This study investigated the ferric chelate reductase (FCR) activity in Azalea under iron deficiency stress, providing valuable insights into the plant's adaptive mechanisms in response to limited iron availability. The results revealed a significant upregulation of FCR

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activity in iron-deficient Azalea plants, accompanied by noticeable declines in chlorophyll content and overall growth. The marked increase in FCR activity observed in iron-deficient Azalea plants aligns with previous studies on other non-graminaceous species, where enhanced FCR activity is a key response to iron scarcity. FCR is responsible for the reduction of Fe(III) to Fe(II), the latter being the more bioavailable form of iron for plant uptake. In conditions where iron availability is low, Azalea plants appear to activate this enzymatic pathway as a compensatory mechanism, thereby enhancing their ability to absorb the necessary iron. This adaptive response is crucial, particularly in alkaline soils where iron predominantly exists in insoluble forms, making it difficult for plants to access.

However, the study's findings also suggest that the upregulation of FCR activity, while beneficial, may not fully compensate for the iron deficiency experienced by the plants. Despite the increase in FCR activity, the iron-deficient Azaleas still exhibited significant chlorosis and reduced growth, indicating that the enzyme's activity may reach a physiological limit beyond which it cannot further alleviate iron deficiency symptoms. The decline in chlorophyll content observed in iron-deficient Azalea plants is a direct consequence of the role of iron in chlorophyll synthesis. Iron is a critical cofactor in the formation of chlorophyll molecules, and its deficiency impairs the biosynthetic pathway, leading to reduced chlorophyll production and the characteristic symptoms of iron chlorosis. The significant reduction in chlorophyll content, as indicated by SPAD readings, reflects the severity of iron deficiency in these plants and its impact on photosynthetic efficiency.

The negative impact of iron deficiency on plant growth, as evidenced by the reductions in shoot length, root length, and biomass, further highlights the essential role of iron in overall plant development. Iron is involved in various physiological processes, including respiration, DNA synthesis, and energy transfer, all of which are crucial for growth. The observed decrease in both shoot and root biomass suggests that iron deficiency not only affects aboveground photosynthetic tissues but also impairs root development, which in turn limits the plant's ability to explore soil and access nutrients and water.

The strong negative correlations between FCR activity and both chlorophyll content and plant growth parameters suggest a complex relationship between the plant's adaptive responses and the physiological effects of iron deficiency. While increased FCR activity represents an attempt by the plant to mitigate the effects of low iron availability, the ongoing deficiency appears to overwhelm the plant's compensatory mechanisms, leading to reduced chlorophyll content and stunted growth. This highlights the limitations of physiological adaptations in the face of severe nutrient deficiencies.

However, it also highlights the challenges posed by iron deficiency, which can lead to significant reductions in plant health and growth despite adaptive responses. Future research could explore additional strategies to enhance iron uptake in Azalea, including the use of soil amendments, iron chelates, or microbial inoculants that promote iron solubilization. These approaches, combined with a deeper understanding of the genetic and biochemical factors influencing FCR activity, could contribute to more effective management of iron deficiency in Azalea and other ornamental plants.

CONCLUSION

This study investigated the response of Azalea to iron deficiency stress, focusing on the activity of ferric chelate reductase (FCR) as a key mechanism for iron

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acquisition. The results demonstrated that under iron-deficient conditions, Azalea significantly upregulates FCR activity in an attempt to enhance the reduction of Fe(III) to Fe(II), thereby improving iron uptake. However, despite this adaptive response, iron deficiency still led to marked reductions in chlorophyll content, pronounced chlorosis, and stunted growth, highlighting the challenges plants face in maintaining optimal physiological function under nutrient stress.

The findings underscore the importance of FCR activity in the iron uptake strategy of Azalea, particularly in environments where iron is not readily available. However, the study also reveals the limitations of this physiological adaptation, as increased FCR activity alone may not fully compensate for the adverse effects of iron deficiency. This suggests that external interventions, such as soil amendments, iron chelates, or foliar iron applications, may be necessary to support the health and growth of Azalea in iron-deficient soils.

Overall, this research contributes to a better understanding of the mechanisms underlying iron acquisition in ornamental plants and provides valuable insights for improving nutrient management practices in horticulture. By addressing iron deficiency more effectively, it may be possible to enhance the ornamental quality and resilience of Azalea, ensuring its continued success in diverse growing environments. Future studies could explore additional strategies to bolster iron uptake and examine the potential for breeding or selecting cultivars with enhanced FCR activity or other iron-efficient traits.

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